Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2023i1.464
*Biol. Clin. Sci. Res. J., Volume, **2023**: 464*
Original Research Article

DISCOVERING THE POTENTIAL IMPACT OF SELENIUM TO ALLEVIATE DROUGHT STRESS IN BARLEY (*HORDEUM VULGARE* L.) VIA PHYSIOLOGICAL INTERFERENCES

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(Received, 10th June 2023, Revised 13th October 2023, Published 15th October 2023)

Abstract *Drought stress is a significant environmental issue that directly impacts plant growth and development by limiting water availability and affecting the overall health of plants. However, innovative solutions are needed to protect plant health and productivity against this significant environmental issue by using all available techniques. By considering this major issue, the pot study aimed to check the potential of foliar application of selenium @ 10mM on various morphological, physiological, and yield aspects of two barley cultivars viz. Jau-17 and Sultan-17 under three different field capacities, including 100%, 75%, and 50% FC, respectively. The results showed maximum plant growth reduction was noticed at 50% FC in both barley cultivars. Interestingly, selenium helped to boost proline content, relative water content (RWC), SPAD chlorophyll, leaf area index (LAI) while it helped to decrease excised leaf water lose (ELWL), membrane thermostability index (MTSI) under drought stress conditions. In addition, various plant morphological and yield-related components were improved by selenium application under drought conditions. Among cultivars, Jau-17 gave the best results in stress tolerance. Conclusively, it is suggested to check the potential of selenium in field conditions under drought stress, especially in rainfed areas*.

Keywords: Drought, Physiology, Cultivars, Osmolytes, Selenium

Introduction

Rapid population growth and climate change threaten worldwide food security. Due to climate change, drought has become the major threat to agrarian production and food security (Kogo et al., 2021). Droughts are increasing worldwide due to less precipitation and altered rainfall patterns. It has been studied by the previous researcher that acute water stress reduces agronomic yields by affecting plant growth, physiology, and reproduction (Farooq et al., 2017). Scientists study that water shortages throughout crop life cycles hinder development and prevent full genetic potential. Most crops are vulnerable to drought stress, mostly through blooming and seed development (Anjum et al., 2017). Even drought-resistant plants suffer from water shortages during the reproductive and seed development stages (Fang et al., 2015). Crops experience drought when they encounter restricted water delivery to their roots or excessive transpiration (Berger et al., 2016). Crops respond to water shortage differently, reliant on the development stage and other circumstances (Berger et al., 2016). When crops were grown in water-limited situations, plant morphology decreased. It has been shown that the conditions of drought result in a decrease in the activity of enzymes, which in turn has a deleterious influence on the production of crops (Kandhol et al., 2022).

Selenium is an element that has been identified as being an essential and useful component for plants (Chauhan et al., 2019). Selenium has been shown to substantially impact crop development, mitigating

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oxidative stress damage, augmenting chlorophyll levels, ameliorating senescence, and enhancing plants' ability to tolerate water stress via the regulation of water status (Khan et al., 2015). Several studies have revealed that selenium has a shielding effect against abiotic stresses (Kapoor et al., 2023). Selenium also has an antioxidant effect because it encourages the manufacture of proline and peroxidase, reducing the amount of intracellular active oxygen species (Pisoschi et al., 2015). Selenium's capacity to standardize the moisture condition of plants results in an increase in the crop's resistance to the negative effects of water scarcity (Hossain et al., 2021). Compared to the situation where no Se treatment was made, the treatment of Se in several crops suffering the consequences of drought resulted in enhanced production and improved water usage productivity (Hossain et al., 2021). The treatment with Se helped improve biomass in cereal seedlings in contrast to the untreated drought treatment (Cipriano et al., 2022). Additionally, Se treatment helped rescue of biochemical enzymes activity and increased the chlorophyll content (Zeeshan et al., 2021).

Barley, a multipurpose crop used for human nourishment and animal fodder, has been domesticated since the early days and continues to play a critical role in modern farming, advancing research in genetics, physiology, pathology, biochemistry, and biotechnology (Harwood et al., 2019). Drought tolerance is crucial for field crops like barley, which ranks fourth among cereals (Kebede et al., 2019). The previous study exposed that the imposition of drought stress caused in a reduction in grain production via the reduction of tiller, spike, and grain numbers per plant, as well as a decrease in individual grain weight in barley (Pecio et al., 2015). Crop irrigation is one of the current drought mitigation measures, however it often causes soil salinization, and breeding approaches for drought-tolerant crops to be labor intensive and complex by relations between genotype and environment (dos Santos et al., 2022). A previous study showed the helpful influence of Selenium in ornamental drought tolerance through the accumulation of complementary solutes and the instigation of enzymes in different agronomic crops (Nagdalian et al., 2023). This experiment aims to explore the influence of selenium (Se) on physiological and biochemical processes in barley, specifically under varying field capacity conditions (Nagdalian et al., 2023). It is hypothesized that adding Se will enhance barley yield and improve its ability to tolerate drought stress by maintaining turgor pressure, accumulating osmoprotectants, and activating antioxidant defense mechanisms (Sehar et al., 2021).

Materials and Methods

At PMAS-AAUR, located at 33.6492°N, 73.0815°E, and 508m above sea level, a pot experiment was conducted during the barley harvest 2021-2022. An earthen pot (22 x 20 cm) containing 8 kg of fine-silt, mixed, scorching vertical, ochraqualfs alluvial loam soil. (USDA). This experiment's primary goal was to determine how selenium applied externally to barley plants at various moisture levels affects the plant's growth. The National Agriculture Research Centre in Islamabad obtained two distinct pedigrees of the cultivars JAU-17 and SULTAN-17. Seeds were surface sterilized with sodium hypochlorite solution (0.1%) and three distilled water rinses. In pots (15 seeds per pot), seeds were germinated, and the necessary emergence data was entered on a record sheet. After the completion of emergence data for further data collection, in each pot, 5 healthy plants were retained, and the rest of them got eliminated by thinning. Upon flag leaf stage, pots were maintained with water equivalent to 100%, 75%, and 50% of field capacity. For the proposed treatment, selenium (Se) was obtained from Sigma Aldrich and sprayed @ 10 mM as foliar application (100%+Se), (75%+Se), (50%+Se) at flag leaf stage. Basal N, P & K were applied to the soil (N: 17 mg, P: 8.5 mg, and K: 8.5 mg kg^{-1} soil). The pots were covered with manually adaptable, flexible, and transparent polyethylene sheets to keep the plants dry and maintain the effects of the treatments. Physical, physiological, and biochemical data described below were recorded and statistically analyzed using STATISTICS 8.1 software according to the CRD 3-way factorial ANOVA.

Relative water content measurement (RWC)

Each pot's relative water content (RWC) was calculated using a second plant leaf. After evaluating the fresh weight (FW), turgid weight (TW) of the leaves and dry weight (DW) of the leaves, which was acquired after 24 hours of drying at 80 °C, was next recorded. Which were acquired after 4 hours of immersing in the distilled water at room temperature under continuous illumination.

$$RWC\ (\%) = \frac{(\text{Fresh weight} - \text{Dry Weight})}{(\text{Turgid Weight} - \text{Dry Weight})} \times 100$$

Membrane thermostability index (MTSI) estimation

Cell membrane stability index (CMSI) was determined by obtaining leaf strips in identical tubes, heated to 100 degrees Celsius for 30 minutes, and then held in a water bath. The following formula was utilized to calculate CMSI:

$$CMSI\ (\%) = \left[1 - \left(\frac{C1}{C2}\right)\right] \times 100$$

C1 is the electrical conductivity of the water in the first set that contained the sample.

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C2 represents the water's electrical conductivity, containing the sample in set two.

ELWL (Excised Leaf Water Losses) calculation

Fresh, fully grown leaves at the flag leaf phase were removed from the source for each replicate treatment to determine the ELWL. Instantaneous fresh weight was measured on an electric weighing scale. The samples were then kept in an incubator at 28 °C and 50% of relative humidity for six hours. Leaf weight was measured followed by dry weight after 24 hours of storage in a hot air oven. ELWL was measured as follows;

$$ELWL = \frac{\text{fresh weight} - \text{weight after 6 hour weight}}{dry\ weight}$$

Measurement of chlorophyll content

A (Konica Minolta Sensing, Inc. Japan, SPAD-502) chlorophyll meter was utilized to gauge the amount of chlorophyll.

Leaf area index (LAI)

An area meter for leaves (CI-202) CID, Inc.) was utilized to measure the leaf area index accurately.

Proline content

Bates et al.'s (1973) technique was employed to determine proline. The study involved homogenizing plant material in 3% sulfosalicylic acid, filtering it, mixing it with glacial acetic and ninhydrin acids, heating it, extracting it with toluene, and measuring its absorbance at 520 nm using a Shimadzu UV 1601 spectrometer. The toluene-containing chromophore was then aspirated and brought to room temperature. Proline in the sample was calculated using appropriate proline standards that were included.

Plant morpho-yield related parameters

Healthy, mature, and well-guarded plants were chosen for growth and morphological traits for parameter at the time of harvest. A measuring tape was used to gauge the height of the plants. The same was done for other plants. Tillers per plant were counted. The number of spikelets per spike followed by spike length was also calculated. Grain yield per plant was also calculated.

Results

Proline

Foliar applied selenium on different barley cultivars under different drought stresses, showing that compatible solutes e.g. proline, have been considerably increased under different drought stresses over control (p≤0.05). The highest proline 225% observed under 50% drought stress over control in both cultivars, as shown in **fig 1**. Interestingly, foliar-applied selenium significantly increased proline in both cultivars under 50% drought stress conditions. Among genotypes, Jau-17 showed the best increment in proline by 46%, while Sultan-17 by 42% over non-treated stressed plants, as shown in **fig 1**.

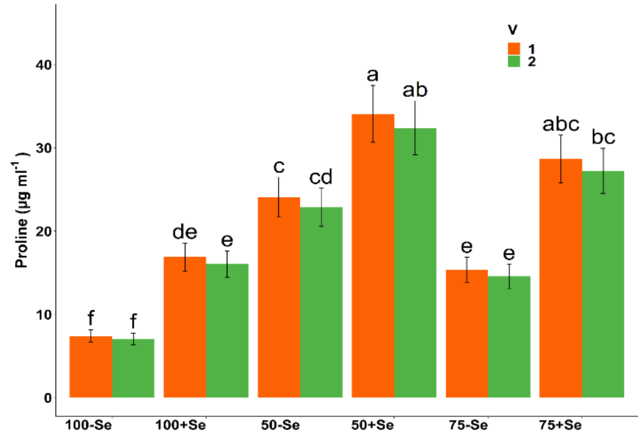

Fig 1. Three field capacities are 100, 75 and 50 with and without Selenium (Se). Varieties are represented by Jau-17 (V1) and Sultan-17 (V2). The graphs are mean value of three replications, and error-bar on each graph are ±S.E. The data was analyzed by three way ANOVA and compared using TUKEY HSD.test (p≤0.05).

Relative water content (RWC)

Results showed that drought stress significantly decreased RWC on barley cultivars over control. The maximum decrease 33% (Jau-17) and 31% (Sultan-17) observed in 50% FC. However, the selenium application helped to increase the RWC over the perspective selenium deficit condition. Among cultivars, RWC was a maximum 20% in Jau-17 and 18% in Sultan-17 over non-selenium stressed plants as shown in **fig 2**.

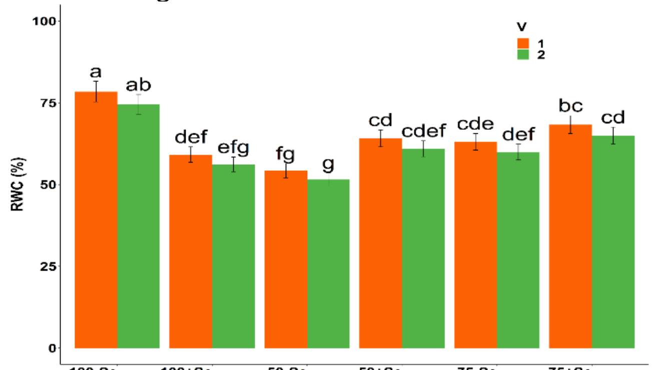

Fig 2. Three field capacities are 100, 75, and 50 with and without Selenium (Se). Varieties are represented by Jau-17 (V1) and Sultan-17 (V2). The graphs are the mean value of three replications, and error-bar on each graph is ±S.E. The data was analyzed by three-way ANOVA and compared using TUKEY HSD test (p≤0.05).

Excised leaf water loses (ELWL)

For ELWL, the maximum 55% in Jau-17 and 58% in Sultan-17 was observed in 50% drought stress over control (p≤0.05). However, the foliar applied selenium significantly decreased the ELWL under 50% drought stress. With Se, the decrease in Jau -17

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was 12%, and in Sultan-17, 15% over Se deficit condition as shown in **fig 3**.

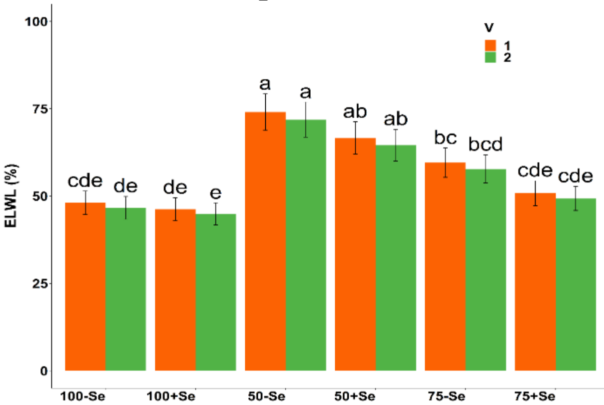

Fig 3. Three field capacities are 100, 75, and 50 with and without Selenium (Se). Varieties are represented by Jau-17 (V1) and Sultan-17 (V2). The graphs are the mean value of three replications, and error-bar on each graph is ±S.E. The data was analyzed by three-way ANOVA and compared by using TUKEY HSD test ($p \leq 0.05$).

Membrane thermostability index (MTSI)

Alike ELWL the MTSI was maximum 88% under 50% drought stress over control in both cultivars ($p \leq 0.05$). The foliar-applied selenium has significantly decreased the MTSI in both genotypes under 50% drought stress. With Se, the Jau-17 and Sultan-17 were to diminished by 66.5% and 65.3%, respectively, over their perspective Se deficit condition as shown in **fig 4**.

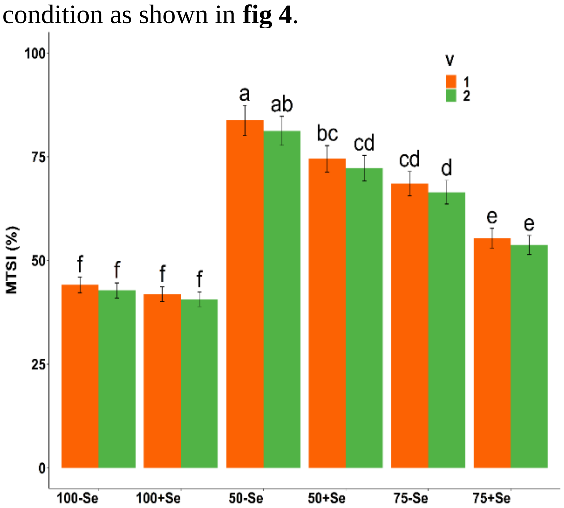

Fig 4. Three field capacities are 100, 75, and 50 with and without Selenium (Se). Varieties are represented by Jau-17 (V1) and Sultan-17 (V2). The graphs are the mean value of three replications, and error-bar on each graph is ±S.E. The data was analyzed by three-way ANOVA and compared using TUKEY HSD test ($p \leq 0.05$).

Spade Chlorophyll

The results showed drought stress significantly decreased the Spade value on both barley cultivars over control. The maximum decrease was 25% and 28% in Jau-17 and Sultan-17 genotypes under 50% FC, respectively. However, the selenium application helped to increase Spade chlorophyll value with 29.22% and 31.64% over the perspective selenium deficit conditions, as shown in **fig 5**.

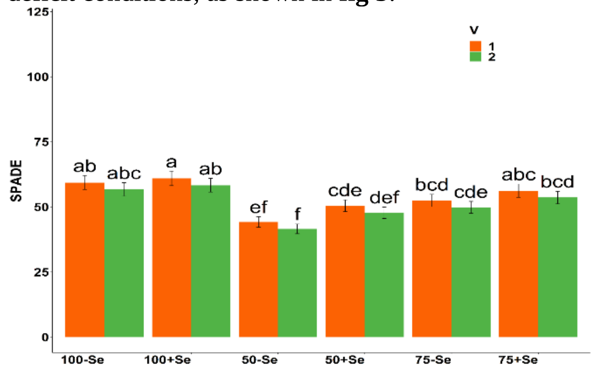

Fig 5. Three field capacities are 100, 75, and 50 with and without Selenium (Se). Varieties are represented by Jau-17 (V1) and Sultan-17 (V2). The graphs are the mean value of three replications, and error-bar on each graph is ±S.E. The data was analyzed by three-way ANOVA and compared using TUKEY HSD test ($p \leq 0.05$).

Leaf area index (LAI)

Data related to LAI showed that drought stress significantly decreased LAI on both barley cultivars over control ($p \leq 0.05$). The maximum decrease is 28% and 32% noticed under 50% FC in Jau-17 and Sultan-17, as shown in Table 1. However, the selenium application helped to increase the 22% and 18% LAI in Jau-17 and Sultan-17 over their perspective selenium deficit condition.

Plant morphological and yield related components

Plant height was significantly decreased by 31% under 50% drought stress level in both barley cultivars compared to the control ($p \leq 0.05$). However, the foliar application of selenium improved plant height by 8% and 6% in Jau-17 and Sultan-17, as shown in Table 1. For tillers, the maximum reduction was noticed by 20% and 28% under 50% drought stress level in both barley cultivars compared to the control ($p \leq 0.05$). Interestingly, foliar application of selenium improved tillers by 8% and 21% in Jau-17 and Sultan-17, as shown in Table 1. Plant yield-related components were also reduced under drought stress compared to control ($p \leq 0.05$). The spikelet's per spike were decreased by 25% in both barley cultivars compared to control ($p \leq 0.05$). However, the foliar application of selenium improved spikelet's per spike by 27% and 19% in Jau-17 and Sultan-17 as shown in Table 1. Data associated with spike length per plant was decreased by 16% and 26% under 50% drought stress over control. Interestingly, the foliar application improved spike length by 28%

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and 20% in Jau-17 and Sultan-17 over their perspective selenium deficit condition, as shown in Table 1. Data related to grain yield per plant was decreased by 38% under 50% drought stress over control. Interestingly, the foliar application improved grain yield per plant by 27% and 11% in Jau-17 and Sultan-17 over their perspective selenium deficit condition, as shown in Table 1.

Table 1. Various plant morphological and yield related components

Cultivars	FC	Se	LAI (cm^2)	Plant length (cm)	No of Tillers $plant^{-1}$	Spike length (cm)	No of Spikelet's $spike^{-1}$	Grains $plant^{-1}$ (g)
Jau-17	100	0	27.12±0.09ab	69.35±1.63c	4.67±0.54a	11.16±0.61e	31.47±1.63d	6.7±1.11ab
		10	29.17±0.45a	70.26±1.65bc	5±0.47a	12.11±0.66ab	33.82±1.75b	6.9±1.08b
	75	0	20.71±0.10d	63.69±1.50g	3±0.47a	9.99±0.55f	28.73±1.49gh	5.8±1.12c
		10	25.01±0.10bc	66.72±1.57e	4±0.47a	11.56±0.63cd	30.40±1.58e	6.2±1.02cd
	50	0	19.66±0.19d	49.69±1.17k	3.33±0.27a	9.28±0.45g	23.62±1.23j	4.1±0.98cd
		10	23.96±0.19c	56.38±1.32i	4±0.47a	11.91±0.54f	30.05±1.46h	5.2±0.94ef
Sultan-17	100	0	27.60±0.25a	70.77±1.66ab	5±0.47a	11.39±0.62de	32.78±1.70c	7.1±1.14bc
		10	29.35±0.26a	71.69±1.68a	5.33±0.72a	12.36±0.68a	35.23±1.83a	6.5±0.96bc
	75	0	23.28±0.10c	64.99±1.53f	4.33±0.72a	10.19±0.56f	29.93±1.55ef	5.1±1.22d
		10	27.58±0.10a	68.08±1.60d	5±0.47a	11.80±0.65bc	31.67±1.64d	6.5±1.14de
	50	0	19.71±0.19d	50.70±1.19j	4±0.47a	8.45±0.46g	24.60±1.28i	4.4±0.94e
		10	24.01±0.19c	57.53±1.35h	4.33±0.72a	10.11±0.55f	29.22±1.52fg	4.9±0.84fg

Three field capacities are 100, 75 and 50 with and without Selenium (Se). Varieties are represented by Jau-17 (V1) and Sultan-17 (V2). The data are mean value of three replications and error-bar on each graph are ±S.E. The data was analyzed by three way ANOVA and compared by using TUKEY HSD.test ($p \le 0.05$).

Discussion

Drought stress profoundly influences plant growth and development by interfering with water intake, resulting in reduced photosynthesis, slowed root growth, and lower crop yields (Ahluwalia et al., 2021). It also causes water-stressed wilting, insufficient nutrient absorption, and hampered reproductive activities, lowering plant vitality and output (Silvestre et al., 2014). Selenium effectively addresses drought stress in plants by enhancing antioxidant defenses, reducing oxidative damage, improving water retention, and leading to healthier, more resilient crops even in water-scarce conditions (Wahab et al., 2022). Proline, an essential osmoprotectant in plants, enhances drought tolerance and cellular integrity by enhancing plants' ability to recover from water shortages (Ghosh et al., 2022). Moreover, stressed plants showed higher proline accumulation, possibly due to proline's crucial role in osmotic control during drought stress (Abdelaal et al., 2021; Amelework et al., 2015). In our research, we found that selenium boosts plant proline levels during drought stress by regulating stress-responsive signaling pathways, as well as promoting increased proline biosynthesis, and maintaining osmotic balance and cellular integrity, thereby enhancing plant health (Ghosh et al., 2022; Raza et al., 2023). Other researchers have also indicated that selenium helps boost proline under drought stress (Ahmad et al., 2016; Ghosh et al., 2022). The membrane thermostability index assesses cell membrane integrity and susceptibility to temperature-induced damage to estimate a plant's stress resistance, particularly under drought stress (Hassan et al., 2021). Djanaguiraman et al. (2018) In the current experiment, Selenium reduces the membrane thermostability index by mitigating oxidative stress and enhancing membrane fluidity (Singhal et al., 2023). This promotes heat tolerance and integrity, bolstering antioxidant systems and reducing lipid peroxidation, ultimately enhancing plant resilience to temperature stress (Hayat et al., 2023). In his examination we additionally found that selenium reduce membrane thermostability index (Karumannil et al., 2023). Drought stress can cause excessive leaf water loss, leading to plant desiccation and disrupting essential physiological processes like photosynthesis and nutrient uptake, compromising plant health (Rao et al., 2016). Likewise, Selenium helps plants reduce excessive leaf water loss by improving stomatal control, which resulted in regulating transpiration rates, and promoting better water use efficiency (Ahmad et al., 2016). Its capacity to do this is attributed to its influence on hormone signaling and antioxidant defense mechanisms at a fundamental physiological level (Mostofa et al., 2021). Ahmad et al., (2016) In his study, under drought conditions, using selenium reduces excessive leaf water loss in plants, helping to enhance drought resistance (Rady et al., 2020). Under drought stress, SPAD chlorophyll readings can either increase due to stress adaptation or decline, depending on the plant's response and stress severity (Hasanuzzaman et al., 2016). Some exceptions were found that Selenium increases chlorophyll concentration in plants by stimulating chlorophyll synthesis and shielding chlorophyll molecules from damage caused by oxidation, through regulating chlorophyll metabolism enzymes (Aliya et al., 2016; Abbas et al., 2016; Zaib et al., 2023). Therefore, this finding also suggests that with the help of selenium, SPAD chlorophyll concentration increases (Naseem et al., 2021). The leaf area index (LAI) is a crucial indicator during drought stress, consequently indicating the extent of foliage and canopy development (Panigrahi et al., 2021). It directly impacts a plant's ability to cope with water scarcity, maintain efficient photosynthesis, Additionally, adapt to water scarcity (Seleiman et al., 2021). Selenium significantly increases the leaf area index in plants by promoting robust growth, consequently optimizing photosynthesis, and mitigating oxidative stress (Rady et al., 2021). Likewise, This deep physiological impact contributes to increased leaf area by enhancing overall leaf area (Pandey et al., 2017). As have been reported previously, Selenium increases the leaf area index in plant. Barley morphology undergoes changes under drought stress, including reduced plant height, shorter leaves, reduced tillering, and smaller grain size, as adaptive responses to conserve water and improve survival, highlighting the importance of water conservation in plant life (Farooq et al., 2012). Selenium enhances barley plant morphology by boosting root and shoot development, modulating hormone signaling pathways, In addition, lowering oxidative stress, Furthermore, resulting in enhanced plant growth and appearance, consequently increasing overall plant structure and appearance (Habibi et al., 2020). Siddiqui et al. (2021) reported that Se application improves the morphology of barley plant. Under drought stress, water scarcity leads to a decrease in seed yield components, including seed quantity per plant and seed size, resulting in reduced crop productivity (Ahanger et al., 2016; Batool et al., 2023). This is primarily due to water scarcity. Selenium significantly enhances seed yield in barley plants by improving flowering, optimizing pollination, and reducing oxidative stress (Ali et al., 2013; 2014; 2016; Ali et al., 2017. This leads to increased seed quantity and size, demonstrating its profound physiological impact on barley plant physiology (Sami et al. 2023; Seleiman et al., 2021). In our study, we found that the SE will be under drought stress and increase the seed yield (Ferdous et al., 2017; Nawaz et al., 2015).

Conclusion

We concluded that selenium is a promising solution for mitigating drought stress in barley plants, improving seed yield, leaf area index, and overall plant morphology. It fine-tunes stress-responsive pathways, stimulates growth, and protects against oxidative damage, offering a significant opportunity for sustainable crop production and global food security. Further research and field trials are needed to harness selenium's potential fully.

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Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Funding

Not applicable

Conflict of Interest

Regarding conflicts of interest, the authors state that their research was carried out independently without any affiliations or financial ties that could raise concerns about biases.

Authors contributions: All authors contribute equally.

[Citation Ali, M.F., Shah, A.U.H., Alvi, M.H., Nawaz, A., Khan, O.U., Iqbal, N., Mahmood, A., Shah, M.T., Ramzan, H,N., Sarwar, M.A. (2023). Discovering the potential impact of selenium to alleviate drought stress in barley (*Hordeum vulgare* l.) via physiological interferences. *Biol. Clin. Sci. Res. J.*, **2023***:* 464. doi: https://doi.org/10.54112/bcsrj.v2023i1.464]